REMARKS

Claims 1-36 were pending in the present application. By virtue of this response, claims 1-24, 26, 27 and 29 have been amended and new claims 37-38 have been added. Accordingly, claims 1-38 are currently under consideration. Amendment and cancellation of certain claims is not to be construed as a dedication to the public of any subject matter of the claims as previously presented.

Applicants are submitting concurrently herewith a Substitute Specification pursuant to 37 C.F.R. 1.125(b) in order to correct an inadvertent word processing error to Greek symbols that occurs throughout the text of the specification of record. The correct notations of α_{1B}-AR and β_2-AR are known to one of skill in the art (see for example page 1, lines 25-30 through page 2, lines 1-7 which describe the family of adrenergic receptors) and are found in the abstract, throughout the text and in certain of the claims. Applicants respectfully request entry of this Substitute Specification.

Pursuant to 37 C.F.R. 1.125(b), Applicants submit that the Substitute Specification includes no new matter. Pursuant to 37 C.F.R. 1.125(b) Applicants are submitting concurrently herewith a marked-up version of the Substitute Specification showing the matter being added to in Double Underlined text and the matter being deleted from as Overstrike text. This marked up version was generated by CompareRite™. Applicants note that CompareRite™ converted the incorrect Greek symbol ϑ to a question mark. In the marked-up version of the Substitute Specification deletion of the Greek symbol ϑ appears as a question mark with an overstrike. Applicants point out that amendments were made to Table 1 (not included in the marked-up version) to correct Greek symbols in the left column.

In the Preliminary Amendment filed by Applicants March 1, 2002, Applicants amended paragraphs of the specification to add sequence identifiers and the amended paragraphs recited the inadvertent word processing errors to Greek symbols. Therefore, in order to clarify the record, Applicants are amending the Substitute Specification to add sequence identifiers.

Support for new claims 37-38 can be found in the specification of record at least at page 17, lines 29-30. Support for the amendment to claim 1 can be found throughout the specification and in particular at page 9, lines 1-2. Support for the amendment to claim 11 can be found throughout the specification and in particular at page 9, lines 10-11. Support for the amendment to claims 3-6 and 13-16 that recites hybridization under high stringency can be found at least at page 12, lines 14-23; page 13, lines 19-22 and at page 15, lines 1-8. Claims 21-22; 24; 26-27; and 29 have been amended for clarity.

Applicants respectfully request entry of the amendments to the claims.

Attached hereto is the Substitute Specification and CompareRite version showing changes between the Substitute Specification and specification of record. A marked-up version of the changes made to the substitute specification and the claims by the current amendment is attached and pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

Concerning the rejection of claims under 35 U.S.C. § 112, first paragraph

A. Claims 1-36 are rejected as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner cites the December 21, 1999 revised written description guidelines for genus/species situations.

Applicants traverse this rejection of claims.

The written description guidelines (see Federal Register: January 5, 2001) state that there is a strong presumption that an adequate written description of the claimed invention is present when the application is filed and that rejection of an original claim for lack of written description should be rare. The Examiner has the initial burden of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the pending claims. The relevant question is whether there is sufficient

written description to inform a skilled artisan that Applicants were in possession of the claimed invention as a whole at the time the application was filed. The Examiner has not met the burden of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the pending claims. One of skill in the art would recognize that the Applicants were in possession of the claimed invention. Therefore, the original claims are in compliance with Section 112, first paragraph written description requirements.

The Examiner at page 2 of the Office action states that "all of the current claims encompass a genus of nucleic acids which are different from those disclosed in the specification". Applicants strongly disagree. The original claims as filed were described in the specification across their full scope. For example, the human α_{1B}-AR (SEQ ID NO:9) and β_2-AR (SEQ ID NO:10) genes are provided in Figures 1 and 2, respectively; primer description is provided at least in the summary of the invention at pages 6-7 and at pages 17-20; at least 8 illustrative primers are described in the specification as SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8; and methods for using the primer pairs are described at least at page 20 through page 28. In addition, conditions for hybridization reactions are described in the specification at least at page 12, lines 5-30 and page 14, lines 9 through page 15, lines 1-20; and conditions for melting temperature are described at least at page 13, lines 3-13. Furthermore, what is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. One of skill in the art at the time of the filing would recognize that the inventor had possession of the claimed invention. Therefore, Applicants are in full compliance with Section 112, first paragraph written description requirements.

Applicants have amended claim 1 and claim 11 for clarity. Claim 1 recites an oligonucleotide primer pair for amplifying a human α_{1B}-adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:9, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is

non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human α_{1B}-adrenergic receptor gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

Claim 11 recites an oligonucleotide primer pair for amplifying a human β_2- adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:10, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human β_2- adrenergic receptor gene shown in SEQ ID NO:10, which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction. The current claims encompass a genus of primer pairs which are described in the specification, the genus has support in the specification and a description of a representative number of species is provided.

The Examiner states that the "essentially identical" language of the claims allows for an indefinite level of variation. Applicants disagree because "essentially identical" is defined in the specification. See page 14, lines 23-30. While disagreeing with the Examiner's assessment, in an effort to expedite prosecution, Claims 1 and 11 recite a primer pair wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:9 and SEQ ID NO:10, respectively. Conditions of high stringency were known to one of skill in the art at the time of the filing and are described in the specification at least at the paragraph bridging pages 14 and 15.

The Examiner states at page 3 of the Office action that the genus is represented in the specification by the particularly named SEQ ID NOS. The written description guidelines, *supra*, state that a claimed genus may be satisfied through sufficient description of a representative number of species by reduction to practice, by reduction to drawings, or by disclosure of relevant identifying characteristics, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the Applicants were in possession of the claimed invention. Functional characteristics and structural features are recited in the claims. Eight illustrative primers are described in the specification as SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, and 8. Furthermore, the description of the genus of claim 1 and claim 11 allow one of skill in the art to define additional species encompassed by the claims. The Examiner states that no common elements or attributes of the sequences are required (Applicants assume the Examiner refers to primers when using the term "sequences"). Applicants invite the Examiner's attention to claim 1 and claim 11 which describe the primer pairs and their structural features. Neither the written description guidelines nor Section 112, first paragraph recite a requirement that "common attributes of sequences" be defined in order to be in compliance. Neither the written description guidelines nor Section 112, first paragraph recite a requirement that specific "alleles, upstream or downstream regions or alternative splice variants" be described in order to be compliant. The relevant analysis for compliance with Section 112, first paragraph written description is whether one of skill in the art at the time of the filing would recognize that the inventor had possession of the claimed invention. Applicants submit that the claims recite and the specification provides sufficient and relevant identifying characteristics of the primers of the present invention such that one of skill in the art would recognize that the inventor had possession of the claimed invention.

The Examiner states at page 3 that the second problem with respect to written description relates to the scope of the diagnosis. The Examiner has not identified to which claims this concern relates. Applicants request clarification on this point.

The Examiner alleges that the specification only discloses association of nocturnal asthma or essential hypertension with the specific glycine 16 polymorphisms in the human alpha-1B adrenergic receptor gene. The Examiner states that the claims do not provide any descriptive support for other diseases associated with this polymorphism. Applicants disagree. The specification at page 2, lines 21-25 describes alterations in the α_{1B}-adrenergic receptor as being associated with disorders such as hypertension, prostatic hypertrophy and malignant arrhythmia in myocardial ischemia. The specification at page 3, lines 14-19 states that research has implicated a causal role of β_2- adrenergic receptor gene in the pathogenesis of asthma and that a variant was noted in hypertension in African Caribbeans. The specification describes at page 8, lines 25-27 that diseases resulting from alterations in the α_{1B}-adrenergic receptor gene and the β_2-adrenergic receptor gene can include cardiovascular, peripheral vascular, pulmonary, prostatic and neuropsychic and endocrine-metabolic disorders.

The Examiner provides no evidence or reasons as to why one of skill in the art would not recognize that the written description of the invention provides support for the pending claims that relate to methods for diagnosing disease.

The Examiner then cites The Regents of the University of California v. Eli Lilly and Co decision by the CAFC and references the structure of the α_{1B}-adrenergic receptor gene. The α_{1B}-adrenergic receptor gene sequence is provided in the specification and is depicted in SEQ ID NO:9. The β_2-adrenergic receptor gene sequence is provided in the specification and is depicted in SEQ ID NO:10. A description of the primers that can amplify these sequences is provided in the specification and claims. Eight illustrative primers are described in the specification.

The Examiner references Fiers v. Sugano and states at the top of page 5 of the Office Action that "the current situation is a definition of the compound solely but its functional utility, as a deletion, without any definition of the particular deletions claimed". Applicants are confused by this statement by the Examiner that relates to deletions and respectfully request clarity from the Examiner as to how this relates to the Section 112, first paragraph, written description rejection of the pending claims.

The Examiner states that there is no record or description which would demonstrate conception of any nucleic acids other than those expressly disclosed. Applicants strongly disagree. The description of the genus of claim 1 and claim 11 allows one of skill in the art at the time of the filing to recognize species encompassed by the claims. One of skill in the art would recognize that Applicants were in possession of the claimed invention and therefore, the claims comply with Section 112, first paragraph written description requirements.

B. Claims 24, 25, 29-31, 33 and 34 stand rejected, allegedly because the specification, while being enabling for diagnosis of nocturnal asthma and essential hypertension by association with the glycine 16 polymorphism, does not reasonably provide enablement for diagnosis of any other disease or the particular diseases with other polymorphisms.

Applicants traverse this rejection. The Examiner states at page 6 of the Office Action that the claims are directed to "methods of diagnosing disease in a subject comprising the steps of determining alleles of the human alpha-1B adrenergic receptor". Applicants invite the Examiner's attention to the claims. Claim 24 recites a method for diagnosing a disease associated with a genetic alteration of a human α_{1B}-adrenergic receptor gene of a subject. Claim 29 recites a method for diagnosing a disease associated with a genetic alteration of a human β_2-adrenergic receptor gene of a subject. One of skill in the art following the teachings of the specification would be able to make and use the claimed invention without undue experimentation. The specification provides disclosure on how to make and use the primer pairs (see for example the specification at page 11 through page 28, line 5); disclosure on how to amplify the α_{1B}-adrenergic receptor gene and β_2- adrenergic receptor gene (see for example the specification at page 20, lines 19 through page 22, line 5) and disclosure on how to make and use the primer pairs for the diagnosis of disease. See for example the specification at page 22, lines 15-24, and at page 32, Example 6 which provides a working example of diagnosis of disease. One of skill in the art would be able to make and use the claimed invention without undue experimentation.

The Examiner states that the claims encompass all diseases. Applicants invite the Examiner's attention to the claims which recite diagnosing a disease <u>associated with a genetic</u>

alteration of the α_{1B}-adrenergic receptor gene or β_2- adrenergic receptor gene. Not all diseases are encompassed by the claims, only those diseases associated with a genetic alteration of this particular gene. Means for assaying for such alterations is provided in the specification at least at page 20, lines 7-13 and page 22, lines 15-24. The Examiner states that no other polymorphisms other than Gly 16 are identified in the specification. Applicants disagree. Gly 16 represents one illustrative polymorphism known to be associated with disease. For example, the specification at page 3, lines 9-14 states that at least 6 polymorphic forms of β_2- adrenergic receptor gene have been identified and include amino acid substitutions at position 16, 27 and 164 which have been implicated in pathogenesis of disease. Furthermore, Buscher et al. (2002, Pharmacogenetics, vol. 12, pages 347-353) disclose the identification of β_2-adrenergic receptor gene polymorphisms in cystic fibrosis lung disease. U.S. Patent 6,498,009 issued December 24, 2002, discloses that polymorphisms in the β_2-adrenergic receptor gene have been shown to be associated with for example congestive heart failure and risk of elevated blood pressure. See the issued claims. The Examiner states that no specific sequences are recited for the specific adrenergic receptor genes. Claims 1 and 11 recite specific SEQ ID NOs for the α_{1B}-adrenergic receptor gene and the β_2-adrenergic receptor gene.

The Examiner states at page 7 that the quantity of experimentation in this area is large. M.P.E.P 2164.06 states that the quantity of experimentation is only one factor involved in determining whether undue experimentation is required. As stated by M.P.E.P 2164.06, an extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine. Applicants submit that sufficient direction and guidance is provided in the specification.

The Examiner at page 7 states that the art is unpredictable and refers to Lavandero et al., Fatemik et al., that relates to beta3 adrenergic receptor, which is not relevant to the subject matter of the instant application (US patent 6,498,00 states at col. 6, lines 33-35 that the β_3 adrenergic receptor is a different molecule than the β_2 adrenergic receptor); Santos et al. and Lin

et al. The Examiner then concludes that it is unpredictable whether any specific allele will be associated with any specific disease. In Lavandero et al., the analysis was performed on 70 Chilean heart failure patients. The author notes that the frequency of β_2 adrenergic receptor (beta2AR) alleles were different to that observed in other Caucasian populations. Santos et al., state that the small number of subjects with the allele encoding Glu27 in the β_2 adrenergic receptor (ADRB2) gene seriously limited the analysis of the association between genotype and phenotype. Lin et al., state that the Gln27Glu beta2-ADR variant might influence body weight. Furthermore, Applicants invite the Examiner's attention to Buscher et al. (2002, Pharmacogenetics, vol. 12, pages 347-353) which disclose the identification of β_2-adrenergic receptor gene polymorphisms in cystic fibrosis lung disease and U.S. Patent 6,498,009 issued December 24, 2002, which discloses that polymorphisms in the β_2-adrenergic receptor gene have been shown to be associated with congestive heart failure and risk of elevated blood pressure. Those of skill in the art at the time of the filing would understand how to make and use the claimed invention without undue experimentation.

The Examiner states that there is one working example. Applicants submit that Section 112, first paragraph does not require any working examples to be present in the specification. Second, the specification provides enabling disclosure for the claimed invention. In particular, Examples 1 and 2 provide generation of specific primer sets. Examples 3 and 4 provide disclosure of uses of the primer sets. Example 5 provides sequence analysis and Example 6 provides disclosure of the use of primer sets for diagnosing diseases associated with genetic alterations in the α_{1B}-adrenergic receptor gene or β_2- adrenergic receptor genes.

The Examiner states at page 8 of the Office Action that the specification does not provide teachings to overcome doubts raised in the art. The Examiner has provided no evidence as to why one of skill in the art would doubt the enablement of the claimed invention. Those of skill in the art at the time of the filing of the patent application would understand how to make and use the claimed invention without undue experimentation.

In view of the arguments above, Applicants respectfully request withdrawal of this Section 112, first paragraph rejection of claims.

Concerning the rejection of claims under 35 U.S.C. § 112, second paragraph

Claims 3-6 and 13-16 are rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants disagree with this rejection. In an effort to expedite prosecution, Applicants have amended these claims thereby obviating the Examiner's rejection.

Concerning the rejection of claims under 35 U.S.C § 102(b)

Claims 1, 2, 11 and 12 stand rejected as allegedly being anticipated by Synaptic Pharmaceutical Corporation (WO 94/08040).

Applicants traverse this rejection.

Applicants point out that WO 94/08040 has no disclosure whatsoever regarding β_2-adrenergic receptor gene. Claims 11 and 12 recite β_2- adrenergic receptor gene. Therefore each and every element of claims 11 and 12 is not present in WO 94/08040. Therefore, the rejection of claims 11 and 12 must fail as a matter of law.

Claim 1 recites an oligonucleotide primer pair for amplifying a human α_{1B}-adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:9, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human α_{1B}-adrenergic receptor gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

WO 94/08040 at page 5, lines 16-21 discloses nucleic acid probes of at least 15 nucleotides and there is no disclosure of the primer pairs as recited in claim 1 or 2. WO 94/08040 at page 55 lines 7-37 disclose PCR primers wherein the sense strand is from nucleotides 567-593 and the antisense strand is from nucleotides 822-847. These primers disclosed in WO 94/08040 anneal to two regions of α_{1B} that are separated by 280 nucleotides in contrast to the presently claimed invention wherein said primer pair anneals to two distinct regions of the human α_{1B}-adrenergic receptor gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides. WO 94/08040 at page 55 lines 34-37 disclose internal probes sense strand is from nucleotides 745-789 and the antisense strand is from nucleotides 770-814. These primers disclosed in WO 94/08040 anneal to two regions of α_{1B} that are separated by 69 nucleotides in contrast to the presently claimed invention wherein said primer pair anneals to two distinct regions of the human α_{1B}-adrenergic receptor gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides. Each and every element of claims 1 and 2 is not contained within WO 94/08040 and therefore this rejection must fail as a matter of law. Applicants respectfully request withdrawal of this Section 102 rejection of claims.

Concerning the rejection of claims under 35 U.S.C § 103(a)

A. Claims 1-14, 16-18, 20 and 21 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Synaptic Pharmaceutical Corporation (WO 94/08040) in view of Ramarao et al. (J. Biol. Chem. (1992) 267(30):21936-21945) and further in view of Emorine et al. (Proc. Natl. Acad. Sci. (1987) 84:6995-6999).

Applicants traverse this rejection of claims. Applicants do not agree or concede that a *prima facie* case of obviousness has been established and submit that the invention is non-obvious in view of the cited references. In order to establish a *prima facie* case of obviousness, there has to be, *inter alia*, some motivation or suggestion provided by the references, or in combination with the knowledge available to the skilled artisan, to modify the art cited or to combine reference teachings. Applicants submit that there is no motivation to combine

references and, even if combined, the combination of references does not produce the claimed invention.

Claim 1 recites an oligonucleotide primer pair for amplifying a human α_{1B}-adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:9, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human α_{1B}-adrenergic receptor gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction. Claims 2-12 are dependent upon claim 1. Claim 21 recites a method for amplifying a segment of a human α_{1B}-adrenergic receptor gene.

Claim 11 recites an oligonucleotide primer pair for amplifying a human β_2- adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:10, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human β_2- adrenergic receptor gene shown in SEQ ID NO:10, which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction. Claims 12-14 and 16-18 and 20 are dependent upon claim 11.

Applicants note that WO 94/08040 has no disclosure or suggestions whatsoever regarding the β_2- adrenergic receptor gene and no disclosure or suggestions whatsoever

regarding identifying polymorphisms in either of the α_{1B}-adrenergic receptor gene or the β_2-adrenergic receptor gene by using primers, much less the presently claimed primer pairs. In contrast to the Examiner's allegation, the primer pairs disclosed in WO 94/08040 at page 55, lines 18-24 would not anneal to two distinct regions about 400 nucleotides apart. The Examiner states that WO 94/08040 teaches methods for diagnosing diseases related to human α_{1B}-adrenergic receptor using nucleic acid probe technology. In fact, WO 94/08040 has no suggestions whatsoever regarding the presently claimed invention. The fact that WO 94/08040 may disclose sequences of the α_{1B}-adrenergic receptor gene in no way renders the presently claimed invention obvious.

Emorine et al. disclose the structure of the gene for human β_2-adrenergic receptor and have no teachings or suggestions whatsoever regarding identifying polymorphisms in the β_2-adrenergic receptor gene by using primers, much less the presently claimed primer pairs. Emorine et al. in no way cure the deficiencies of WO 94/08040. Ramarao et al. disclose the genomic organization and expression of the human α_{1B}-adrenergic receptor and have no teachings or suggestions whatsoever regarding identifying polymorphisms in the α_{1B}-adrenergic receptor by using primers, much less the presently claimed primer pairs. Ramarao et al. in no way cure the deficiencies of WO 94/08040. There is no motivation to combine references and if combined one of skill in the art would not arrive at the presently claimed invention. The presently claimed invention is non-obvious over the cited art and Applicants request withdrawal of this Section 103 rejection of claims.

B. Claims 1, 2, 11, 12 and 21-36 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Synaptic Pharmaceutical Corporation (WO 94/08040) in view of Cotton et al. (Current Opinion in Biotechnol (1992) 3:24-30).

Applicants traverse this rejection of claims. The deficiencies of WO 94/08040 have been discussed above. WO 94/08040 has no teachings or suggestions of identifying polymorphisms in either of the α_{1B}-adrenergic receptor gene or the β_2- adrenergic receptor gene by using primer pairs, much less the presently claimed primer pairs, and in fact has no disclosure whatsoever of

the β_2-adrenergic receptor gene. Cotton et al. relate to detection of mutants in DNA. Cotton et al. have no teachings whatsoever regarding either of the α_{1B}-adrenergic receptor gene or the β_2-adrenergic receptor gene. That the Examiner would combine these references to allege non-obviousness is the result of the impermissible use of hindsight reconstruction. The presently claimed invention is non-obvious over the cited art. Applicants respectfully request withdrawal of the Section 103(a) rejection of claims.

CONCLUSION

Applicants have made a sincere effort to overcome the rejections and address all issues that were raised in the outstanding Office Action. Accordingly, reconsideration and allowance of the pending claims are respectfully requested. If it is determined that a telephone conversation would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 220002058901.

Respectfully submitted,

Dated: March 5, 2003

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Applicants are submitting concurrently herewith a Substitute Specification to correct inadvertent word processing errors to Greek symbols that occur throughout the specification of record. Also submitted concurrently herewith is a marked up version of the Substitute Specification generated by CompareRite with additions showing as Double Underlined text and deletions as Overstrike text.

The paragraph [0023] of the Substitute Specification has been amended as follows:

(Amended)

Figure 5 **(SEQ ID NO:11)** depicts the sequence profile of fragment A of the human α_{1B}-AR gene. The amplified fragments were sequenced with the amplification,primer SEQ ID NO: 1 using Applied Biosystems' DNA sequencer. Each peak indicates a different nucleotide. In some cases "ambiguous reads" (N) occur as the result of duplicate bases (heterozygosity) or reading ambiguities, which can generally be corrected afterwards.

The paragraph [0024] of the Substitute Specification has been amended as follows:

(Amended)

Figure 6 **(SEQ ID NO:12)** depicts the sequence profile of fragment A of the human $ß_2$-AR gene. The amplified fragments were sequenced with the amplification primer SEQ ID NO:6 using Applied Biosystems' DNA sequencer. Each peak indicates a different nucleotide. In some cases "ambiguous reads" (N) occur as the result of duplicate bases (heterozygosity) or reading ambiguities, which can generally be corrected afterwards.

Paragraph [0068] of the Substitute Specification has been amended as follows:

(Amended)

In a preferred embodiment, the method is used for diagnosing nocturnal asthma based on glycine 16 polymorphism in β_2-AR gene. In another embodiment, the method is used for a diagnosis of essential hypertension based on the same genetic polymorphism. While assaying for glycine 16 polymorphism, genomic DNA of the test subject can be obtained from a blood sample. The N-terminal fragment A (as shown in Figure 2) that encompasses sequences encoding the residue glycine 16 can be amplified using primers SEQ ID NOS:5 and 6, or derivatives thereof. Direct sequencing of the amplified products using the same primers employed in the amplification procedure may be performed to detect the single base change (adenosine to guanosine) at position 46 (numbering from the start codon) that results in amino acid substitution of arginine with glycine. Homozygosity of glycine 16 indicates the presence or a predisposition to nocturnal asthma and/or essential hypertension. Glycine 16 homozygotes will be apparent by comparing the sequence peaks from the subject sample to those of the controls (for an example of the sequence output from an automated DNA sequencer, see Figure 5 (SEQ ID NO:11) or 6 (SEQ ID NO:12)). A single peak corresponding to 27 nucleotide guanosine at position 46 is indicative of glycine 16 homozygosity. Two overlapping peaks, each representing guanosine or adenosine respectively at position 46, suggest heterozygosity of glycine 16. Finally, a single peak representing adenosine shows that the subject is homozygous in arginine 16.

Paragraph [0076] of the Substitute Specification has been amended as follows:

(Amended)

Example 5

Sequence Analysis

The PCR products of expected sizes were cut from the gel and the DNA was purified using QIAquick Gel Extraction Kit. The extracted DNA was resuspended in Tris-EDTA buffer (10mM Tris-Cl, 1mM EDTA, pH 8. 0) and concentrated using a Centricon Concentrator (Amicon). The purified gene fragments were then sequenced by an automated DNA sequencer (Applied Biosystems, model 377) using one or more of the same primers employed in PCR. The upstream primer SEQ ID NO: 1 and the downstream primer SEQ ID NO: 6 were used for sequencing the amplified products, fragment A of the human α_{1B}-adrenergic receptor gene and fragment A of β_2-adrenergic receptor gene, respectively. As shown in Figure 5 (SEQ ID NO:11) and 6 (SEQ ID NO:12), each sequencing read approximately 550 bases. Other primers described herein including primer SEQ ID NOS: 1, 2, 3, 5, and 7 can also be used for direct sequencing with high reliability. By use of an automated sequencer and sequencing PCR products from in excess of 15 different subjects, we obtained consistent results in accordance with the published coding sequences of the human β_2- and exon 1 of the human α_{1B}-adrenergic receptor. Repeated sequencing of PCR products of the same individuals revealed a 100% reliability of our PCR methods without requirement for repeat isolation of PCR fragments. Occasionally occurring "ambiguous reads", which are the result of a reading error of the automated sequencer, can generally be corrected afterwards without re-isolating and sequencing the PCR fragments (Figures 5 and 6).

In the Claims:

Claims 1-24, 26, 27 and 29 have been amended, as follows:

1. (Once Amended) An oligonucleotide primer pair for amplifying a human α_{1B}-adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:9, is non-self hybridizing, [contains at least 15 nucleotides] has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human α_{1B}-adrenergic receptor

gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of [the] said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

2. (Once Amended) An oligonucleotide primer pair of claim 1, wherein said primer pair amplifies a fragment selected from the group consisting of[:] region A in Figure 1, and region B in Figure 1.

3. (Once Amended) An oligonucleotide primer pair of claim [2] 1, wherein each individual primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

4. (Once Amended) An oligonucleotide primer pair of claim [2] 1, wherein at least one primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No: 1 5'CGGGGGAAGCAAAGTTTCA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

5. (Once Amended) An oligonucleotide primer pair of claim [2] 1 wherein each individual primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

6. (Once amended) An oligonucleotide primer pair of claim [2] 1, wherein at least one primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

7. (Once Amended) An oligonucleotide primer pair of claim [2] 1 [having] comprising the nucleotide sequences SEQ ID No: 1 5'CGGGGGAAGCAAAGTTTCA3' and SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

8. (Once Amended) An oligonucleotide primer pair of claim [2] 1, wherein at least one primer of said pair [has] comprises the nucleotide sequence SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

9. (Once Amended) An oligonucleotide primer pair of claim [2] 1 [having] comprising the nucleotide sequences SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' and SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

10. (Once Amended) An oligonucleotide primer pair of claim [2] 1, wherein at least one primer of said pair [has] comprises the nucleotide sequence SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

11. (Once amended) An oligonucleotide primer pair for amplifying a human β_2-adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID

NO:10, is non-self hybridizing, [contains at least 15 nucleotides,] has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human β_2- adrenergic receptor gene shown in SEQ ID NO:10, which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of [the] said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

12. (Once Amended) An oligonucleotide primer pair of claim 11, wherein said primer pair amplifies a fragment selected from the group consisting of[:] region A in Figure 2; and region B in Figure 2.

13. (Once Amended) An oligonucleotide primer pair of claim [12] 11, wherein each individual primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

14. (Once Amended) An oligonucleotide primer pair of claim [12] 11, wherein at least one primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No: 5 5'GAATGAGGCTTCCAGGCGTC3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

15. (Once Amended) An oligonucleotide primer pair of claim [12] 11, wherein each individual primer of said pair comprises a linear sequence [essentially identical] that hybridizes

under high stringency to the polynucleotide shown in SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or a linear sequence that hybridizes under high stringency to the polynucleotide show in SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

16. (Once Amended) An oligonucleotide primer pair of claim [12] 11, wherein at least one primer of said pair comprises a linear sequence [essentially identical] that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

17. (Once Amended) An oligonucleotide primer pair of claim [12] 11 [having] comprising the nucleotide sequences SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' and SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

18. (Once Amended) An oligonucleotide primer pair of claim [12] 11, wherein at least one primer of said pair [has] comprises the nucleotide sequence SEQ ID No: 5 5'GAATGAGGCTTCCAGGCGTC3' or SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

19. (Once Amended) An oligonucleotide primer pair of claim [12] 11 [having] comprising the nucleotide sequences SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' and SEQ ID No: 8 5'TCCTCTAGGACTAAAGCTC3'.

20. (Once Amended) An oligonucleotide primer pair of claim [12] 11, wherein at least one primer of said pair [has] comprises the nucleotide sequence SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

21. (Once Amended) A method of amplifying a segment of a human [I]$\underline{\alpha_{1B}}$-adrenergic receptor gene of a subject comprising the step[s] of:

[a) providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the <u>human α_{1B}-adrenergic receptor</u> gene <u>from nucleic acid contained within a biological sample</u> by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene.

22. (Once Amended) A method for identifying a genetic variation in a human [I]$\underline{\alpha_{1B}}$-adrenergic receptor gene of a subject comprising the steps of:

a) [providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the a human [I]$\underline{\alpha_{1B}}$-adrenergic receptor gene <u>from nucleic acid contained within a biological sample</u> by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene; and

[c)]<u>b)</u> identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step.

23. (Once Amended) A method for identifying a genetic variation in a human [I]$\underline{\alpha_{1B}}$-adrenergic receptor gene of a subject according to claim 22, wherein the sequence analytical step is selected from the group of nucleotide sequencing, single-strand conformation polymorphism assay, allele-specific oligonucleotide hybridization, Southern blot analysis, and restriction endonuclease digestion.

24. (Once Amended) A method for diagnosing a disease associated with a genetic alteration of a human α_{1B}-adrenergic receptor gene of a subject comprising the steps of:

a) [providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the [I]α_{1B}-adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene;

[c)]b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step; and

[d)]c) determining a correlation of the detected variation between the subject and a control.

26. (Once Amended) A method of amplifying a segment of a human β_2-adrenergic receptor gene of a subject comprising the step[s] of:

a) [providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the human β_2-adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene.

27. (Once Amended) A method for identifying a genetic variation in a human β_2-adrenergic receptor gene of a subject comprising the steps of:

a) [providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the human β_2-adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene; and

[c)]b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step.

29. (Once Amended) A method for diagnosing a disease associated with a genetic alteration of a human β_2-adrenergic receptor gene of a subject comprising the steps of:

a) [providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the human β_2-adrenergic receptor gene encoding said receptor from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene;

[c)]b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step; and

[d)]c) determining a correlation of the detected variation between the subject and a control.

New claims 37-38 have been added.